ALLOWED CLAIMS/ TJ

1. (Amended) A method for sequencing at least a portion of a RNA involving converting the RNA to a DNA and simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of

wherein one of said at least two thermostable DNA polymerases has reverse transcriptase activity,

to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers;

(b) separating at least said truncated copies to make a sequence ladder; and thereafter

(c) reading the sequence ladder to obtain the sequence of said at least a portion of said RNA wherein the conversion of the RNA to the DNA is conducted in the presence of the at least two thermostable DNA polymerases.

2. The method of claim 1, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

3. The method of claim 1, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

4. The method of claim 1, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

5. (Amended) The method of claim 4, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation [or a functional derivative thereof].

6. (Amended) The method of claim 5, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™, or Thermo Sequenase™ [or functional derivatives thereof].

7. (Amended) The method of claim 6, wherein said first thermostable DNA polymerase is Thermo Sequenase™ [or a functional derivative thereof].

8. The method of claim 1, wherein said second thermostable DNA polymerase has reverse transcriptase activity.

9. (Amended) The method of claim 8, wherein said second thermostable DNA polymerase is Taq DNA polymerase, [or a functional derivative thereof,] Tth DNA polymerase, [or a functional derivative thereof,] Tfl DNA polymerase, [or a functional derivative thereof, or] Klentaq (Taq DNA polymerase)(-exo5'-3')[, or a functional derivative thereof,] or a DNA polymerase from Carboxydothermus hydrogenoformans having reverse transcriptase activity[, or a functional derivative of the DNA polymerase].

10. The method of claim 9, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase or Tfl DNA polymerase.

11. (Amended) The method of claim 1, wherein said first thermostable DNA polymerase is Thermo Sequenase™ [, or a functional derivative thereof,] and said second thermostable DNA polymerase is Taq DNA polymerase[, or a functional derivative thereof].

12. (Amended) The method of claim 11, wherein said second thermostable DNA polymerase is Tth DNA polymerase[, or a functional derivative thereof,] and wherein step (a) is carried out in the presence of $MnCl_2$ or Mn acetate.

13. The method of claim 1, wherein the thermocycling reaction in step (a) is carried out without interruption in a single container, vessel or tube.

14. The method of claim 1, wherein the ratio of said first primer to said second primer is not equal to 1:1.

15. The method of claim 14, wherein said ratio is between about 2:1 and about 3:1.

16. The method of claim 15, wherein said ratio is about 2:1.

17. The method of claim 1, wherein the first and second primers are differently labelled.

18. The method of claim 1, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least 55°C.

19. The method of claim 1, wherein said RNA in said mixture is a RNA of a single-copy gene.

20. The method of claim 1, wherein said mixture further comprises at least one thermostable pyrophosphatase.

21. The method of claim 1, wherein at least one of the first and second primers has a length that, in combination with a high annealing temperature, prevents annealing to unspecific DNA fragments during the heat denaturation of the thermocycling reaction.

22. The method of claim 21, wherein said length is at least 18 nucleotides.

23. The method of claim 1, wherein said RNA in said mixture is obtained from a body fluid, hairs, a cell, cells or fractions thereof, a tissue or fractions thereof, cell cultures or fractions thereof, bacteria or viruses.

24. The method of claim 1, wherein said RNA in said mixture is unpurified RNA.

25. The method of claim 24, wherein said RNA is total genomic RNA.

26. The method of claim 1, wherein said RNA in said mixture is a RNA of a single-copy gene and said mixture further comprises genomic DNA.

27. The method of claim 1, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 55°C.

28. The method of claim 27, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 68°C.

29. The method of claim 1, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 100:1 and about 1000:1.

30. The method of claim 29, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 300:1 and about 600:1.

31. The method of claim 1, wherein said deoxynucleotides or deoxynucleotide derivatives are present at a concentration of about 300 μM to about 2 mM.

32. The method of claim 1, wherein said at least one dideoxynucleotide or another terminating nucleotide is present at a concentration of about 1 μM to about 5 μM.

33. The method of claim 1, wherein the length of said DNA is at least 500 nucleotides between the 3' ends of the first and second primers.

34. The method of claim 1, wherein said mixture further comprises at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

35. The method of claim 34, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

36. The method of claim 34, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

37. (Amended) A kit for sequencing at least a portion of a RNA, comprising deoxynucleotides or deoxynucleotide derivatives, which deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP;

at least one dideoxynucleotide or another terminating nucleotide; and

at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide in comparison to said first thermostable DNA polymerase, wherein at least one of said at least two thermostable DNA polymerases has reverse transcriptase activity;

wherein the at least two DNA polymers are mixed so that conversion of the RNA to the DNA will be conducted in the presence of the at least two thermostable DNA polymerases.

38. The kit of claim 37, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

39. The kit of claim 37, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

40. The kit of claim 37, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

41. (Amended) The kit of claim 40, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation [or a functional derivative thereof].

42. (Amended) The kit of claim 41, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™, or ThermoSequenase™ [or functional derivatives thereof].

43. (Amended) The kit of claim 42, wherein said first thermostable DNA polymerase is ThermoSequenase™ [or a functional derivative thereof].

44. (Amended) The kit of claim 37, wherein said second thermostable DNA polymerase is Taq DNA polymerase, [or a functional derivative thereof,] Tth DNA polymerase, [or a functional derivative thereof,] Tfl DNA polymerase, [or a functional derivative thereof, or] Klentaq (Taq DNA polymerase)(-exo5'-3')[, or a functional derivative therefo,] or a DNA polymerase from Carboxydothermus hydrogenoformans having reverse transcriptase activity[, or a functional derivative of the DNA polymerase]].

45. (Amended) The kit of claim 44, wherein said second thermostable DNA polymerase is Taq DNA polymerase[, or functional derivative thereof].

46. The kit of claim 37, further comprising $MnCl_2$ or Mn acetate, wherein said second thermostable DNA polymerase is Tth DNA polymerase.

47. The kit of claim 37, wherein the ratio of said first primer to said second primer is not equal to 1:1.

48. The kit of claim 47, wherein said ratio is between about 2:1 and about 3:1.

49. The kit of claim 48, wherein said ratio is 2:1.

50. The kit of claim 37, further comprising at least one thermostable pyrophosphatase.

51. The kit of claim 37, further comprises at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at

least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

52. The kit of claim 51, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

53. The kit of claim 51, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

54. The kit of claim 51, wherein said agent is an acid anhydride.

55. The kit of claim 54, wherein said agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

56. The kit of claim 51, wherein said agent is a compound having at least one acid anhydride group per molecule.

57. The kit of claim 56, wherein said agent is a compound having two acid anhydride groups per molecule.

58. The kit of claim 57, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

59. The method of claim 34, wherein said agent is an acid anhydride.

60. The method of claim 59, wherein said agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

61. The method of claim 34, wherein said agent is a compound having at least one acid anhydride group per molecule.

62. The method of claim 61, wherein said agent is a compound having two acid anhydride groups per molecule.

63. The method of claim 62, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

64. (Amended) A method for sequencing at least a portion of a DNA involving simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of

(a) subjecting a mixture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat denaturation, annealing and synthesis, wherein said mixture comprises

said DNA,

a buffer solution,

a first primer which is able to hybridize with a strand of said DNA,

a second primer which is able to hybridize with a strand of said DNA complementary to the strand with which the first primer is able to hybridize, wherein at least one of the first and second primers is labelled,

deoxynucleotides or deoxynucleotide derivatives, wherein said deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP,

at least one dideoxynucleotide or another terminating nucleotide,

at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase,

which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, and

at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, [wherein said at least one polymerase-inhibting agent is a compound having at least one acid anhydride group per molecule,]

to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers;

(b) separating at least said truncated copies to make a sequence ladder; and thereafter

(c) reading the sequence ladder to obtain the sequence of said at least a portion of said DNA.

65. The method of claim 64, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

66. The method of claim 64, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

67. The method of claim 64, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

68. (Amended) The method of claim 67, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation [or a functional derivative thereof].

69. (Amended) The method of claim 68, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™, or Thermo Sequenase™ [or functional derivatives thereof].

70. (Amended) The method of claim 69, wherein said first thermostable DNA polymerase is Thermo Sequenase™ [or a functional derivative thereof].

71. (Amended) The method of claim 64, wherein said at least one polymerase-inhibiting agent has at least one acid anhydride group per molecule [second thermostable DNA polymerase has reverse transcriptase activity].

72. (Amended) The method of claim 64 [71], wherein said second thermostable DNA polymerase is Taq DNA polymerase, [or a functional derivative thereof,] Tth DNA polymerase, [or a functional derivative thereof,] Tfl DNA polymerase, [or a functional derivative thereof,] or Klentaq (Taq DNA polymerase)(-exo5'-3')[, or a functional derivative thereof].

73. The method of claim 72, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase or Tfl DNA polymerase.

74. (Amended) The method of claim 64, wherein said first thermostable DNA polymerase is Thermo Sequenase™ [, or a functional derivative thereof,] and said second thermostable DNA polymerase is Taq DNA polymerase[, or a functional derivative thereof].

75. (Amended) The method of claim 74, wherein said second thermostable DNA polymerase is Tth DNA polymerase[, or a functional derivative thereof,] and wherein step (a) is carried out in the presence of $MnCl_2$ or Mn acetate.

76. The method of claim 64, wherein the thermocycling reaction in step (a) is carried out without interruption in a single container, vessel or tube.

77. The method of claim 64, wherein the ratio of said first primer to said second primer is not equal to 1:1.

78. The method of claim 77, wherein said ratio is between about 2:1 and about 3:1.

79. The method of claim 78, wherein said ratio is about 2:1.

80. The method of claim 64, wherein the first and second primers are differently labelled.

81. The method of claim 64, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least 55°C.

82. The method of claim 64, wherein said DNA in said mixture is a single-copy DNA in a complex mixture of DNA.

83. The method of claim 64, wherein said mixture further comprises at least one thermostable pyrophosphatase.

84. The method of claim 64, wherein at least one of the first and second primers has a length that, in combination with a high annealing temperature, prevents annealing to unspecific DNA fragments during the heat denaturation of the thermocycling reaction.

85. The method of claim 84, wherein said length is at least 18 nucleotides.

86. The method of claim 64, wherein said DNA in said mixture is obtained from a body fluid, hairs, a cell, cells or fractions thereof, a tissue or fractions thereof, cell cultures or fractions thereof, bacteria or viruses.

87. The method of claim 64, wherein said DNA in said mixture is unpurified DNA.

88. The method of claim 87, wherein said DNA is total genomic DNA.

89. The method of claim 64, wherein said DNA in said mixture is a single-copy DNA, wherein said mixture further comprises total genomic DNA.

90. The method of claim 64, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 55°C.

91. The method of claim 90, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 68°C.

92. The method of claim 64, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 100:1 and about 1000:1.

93. The method of claim 92, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 300:1 and about 600:1.

94. The method of claim 64, wherein said deoxynucleotides or deoxynucleotide derivatives are present at a concentration of about 300 μM to 2 mM.

95. The method of claim 64, wherein said at least one dideoxynucleotide or another terminating nucleotide is present at a concentration of about 1 to 5 μM.

96. The method of claim 64, wherein the length of the DNA in said mixture is at least 500 nucleotides between the 3' ends of the first and second primers.

97. The method of claim 64, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

98. The method of claim 64, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

99. (Amended) The method of claim 64, wherein said at least one polymerase-inhibiting [polymerase-inhibting] agent reversibly loses inhibitory activity at the temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, thereby enabling said agent to inhibit said at least one of said at least two thermostable DNA polymerases in more than one thermocycle.

100. (Amended) A kit for sequencing at least a portion of a DNA [RNA], comprising

deoxynucleotides or deoxynucleotide derivatives, which deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP;

at least one dideoxynucleotide or another terminating nucleotide;

at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA

polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide in comparison to said first thermostable DNA polymerase; and

at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule[, wherein said at least one polymerase-inhibting agent is a compound having at least one acid anhydride group per molecule].

101. The kit of claim 100, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

102. The kit of claim 100, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

103. The kit of claim 100, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

104. (Amended) The kit of claim 103, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation [or a functional derivative thereof].

105. (Amended) The kit of claim 104, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™, or ThermoSequenase™ [or functional derivatives thereof].

106. (Amended) The kit of claim 105, wherein said first thermostable DNA polymerase is ThermoSequenase™ [or a functional derivative thereof].

107. (Amended) The kit of claim 100, wherein said second thermostable DNA polymerase is Taq DNA polymerase, [or a functional derivative thereof,] Tth DNA polymerase, [or a functional derivative thereof,] Tfl DNA polymerase, [or a functional derivative thereof, or] Klentaq (Taq DNA polymerase)(-exo5'-3'),[or a functional derivative therefo,] or a DNA polymerase from Carboxydothermus hydrogenoformans having reverse transcriptase activity[, or a functional derivative of the DNA polymerase].

108. (Amended) The kit of claim 107, wherein said second thermostable DNA polymerase is Taq DNA polymerase[, or functional derivative thereof].

109. The kit of claim 100, further comprising $MnCl_2$ or Mn acetate, wherein said second thermostable DNA polymerase is Tth DNA polymerase.

110. The kit of claim 100, wherein the ratio of said first primer to said second primer is not equal to 1:1.

111. The kit of claim 110, wherein said ratio is between about 2:1 and about 3:1.

112. The kit of claim 111, wherein said ratio is about 2:1.

113. The kit of claim 100, further comprising at least one thermostable pyrophosphatase.

114. The kit of claim 100, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

115. The kit of claim 100, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

116. The kit of claim 100, further comprises an antibody against one of said at least two thermostable DNA polymerases, wherein said antibody loses inhibitory ability, thereby allowing said one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

117. The kit of claim 116, wherein inhibition of said one of said at least two thermostable DNA polymerases by said antibody begins at a lower temperature than inhibition of said at least one of said at least two thermostable DNA polymerases by said polymerase-inhibiting agent.

118. The kit of claim 100, wherein said polymerase-inhibiting agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

119. The kit of claim 100, wherein said polymerase-inhibiting agent is a compound having two acid anhydride groups per molecule.

120. The kit of claim 119, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

121. (Amended) The kit of claim 100, wherein said at least one polymerase-inhibiting [polymerase-inhibting] agent reversibly loses inhibitory activity at the temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, thereby enabling said agent to inhibit said at least one of said at least two thermostable DNA polymerases in more than one thermocycle.

122. The method of claim 64, wherein said mixture further comprises an antibody against one of said at least two thermostable DNA polymerases, wherein said antibody loses inhibitory ability, thereby allowing said one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

123. The method of claim 122, wherein inhibition of said one of said at least two thermostable DNA polymerases by said antibody begins at a lower temperature than inhibition of said at least one of said at least two thermostable DNA polymerases by said polymerase-inhibiting agent.

124. (Amended) The method of claim 71 [64], wherein said polymerase-inhibiting agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

125. (Amended) The method of claim 71 [64], wherein said agent is a compound having two acid anhydride groups per molecule.

126. The method of claim 125, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

132. (New) The method of claim 64, wherein said mixture further comprises at least one agent that lowers the melting point of the DNA.

133. (Once Amended) The method of claim 132, wherein said at least one agent is selected from the group consisting of glycerin, trehalose, betaine and [or] DMSO.

134. (New) The method of claim 97, wherein an inhibitory activity of said at least one polymerase-inhibiting agent is reversibly reduced at a specific temperature and after a specific number of thermocycles allowing sequencing of the DNA to start after the DNA has been amplified.

135. (New) The method of claim 134, wherein the inhibitory activity of said at least one polymerase-inhibiting agent is reversibly reduced when the reaction mixture is exposed at an elevated temperature.

136. (New) The method of claim 135, wherein said first thermostable DNA polymerase is a DNA polymerase which carries a Tabor-Richardson mutation and has no 5' to 3' exonuclease activity.

137. (New) The method of claim 136, wherein said first thermostable DNA polymerase is selected from the group consisting of AMPLITAQ FS™, TAQUENASE™, and THERMOSEQUENASE.

138. (New) The kit of claim 100 further comprising a polymerase-inhibiting agent X against one of said at least two thermostable DNA polymerases, wherein said polymerase-inhibiting agent X and said at least one polymerase-inhibiting agent can inhibit different thermostable DNA polymerases.

139. (New) The kit of claim 138, wherein said polymerase-inhibiting agent X can inhibit said second thermostable DNA polymerase and said at least one polymerase-inhibiting agent can inhibit said first thermostable DNA polymerase.

140. (New) The kit of claim 138, wherein said polymerase-inhibiting agent X is an antibody against said second thermostable DNA polymerase.

141. (New) The kit of claim 100 further comprising at least one agent that lowers the melting point of the DNA.

142. (Once Amended) The kit of claim 141, wherein said at least one agent is selected from the group consisting of glycerin, trehalose, betaine and [or] DMSO.

143. (New) The method of claim 8, wherein said mixture further comprises a polymerase-inhibiting agent against said second thermostable DNA polymerase.

144. (New) The method of claim 143, wherein an inhibitory activity of said polymerase-inhibiting agent is reduced after reverse transcription of the RNA. --

--145. The kit of claim 124 wherein said polymerase-inhibiting agent is citraconic anhydride.--

146. The method of claim 64 further comprising, prior to step (a), a step of mixing all of said DNA, said buffer solution, said first primer, said second primer, said deoxynucleotides or deoxynucleotide derivatives, said dideoxynucleotide or other terminating nucleotide, the at least two thermostable DNA polymerases and the at least one polymerase-inhibiting agent in a single container, vessel or tube.